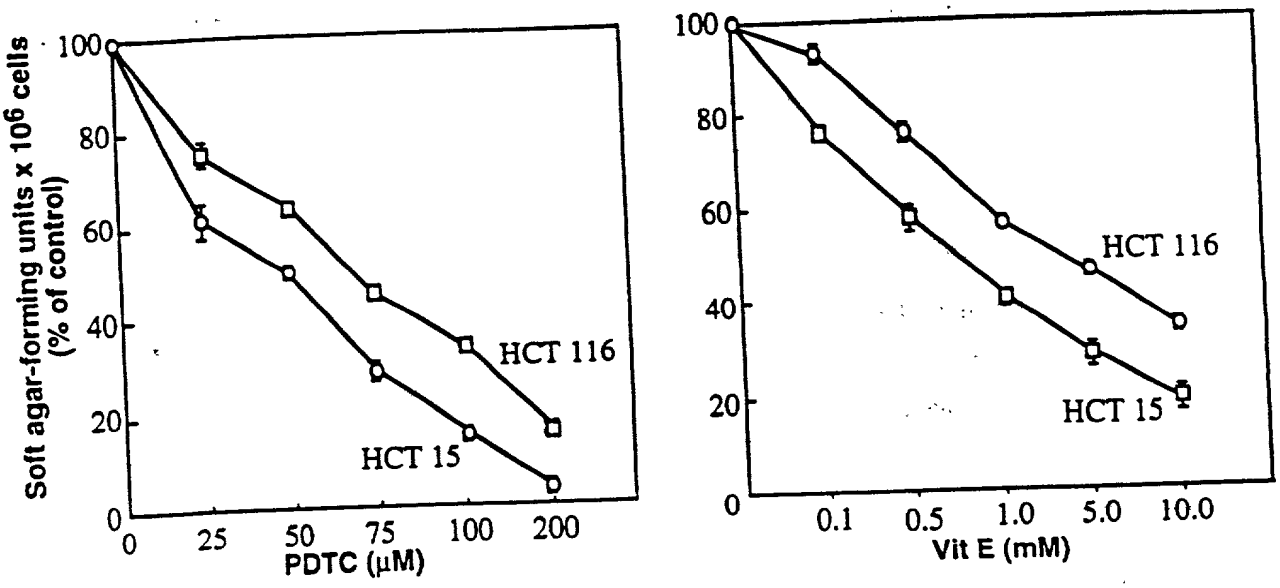

Soft agar-forming units x 10^6 cells
(% of control)
100
80
60
40
20
0
0
25
50
75
100
200
PDTC (μM)
HCT 116
HCT 15
100
80
60
40
20
0
0.1
0.5
1.0
5.0
10.0
Vit E (mM)
HCT 116
HCT 15

FIGURE 1A

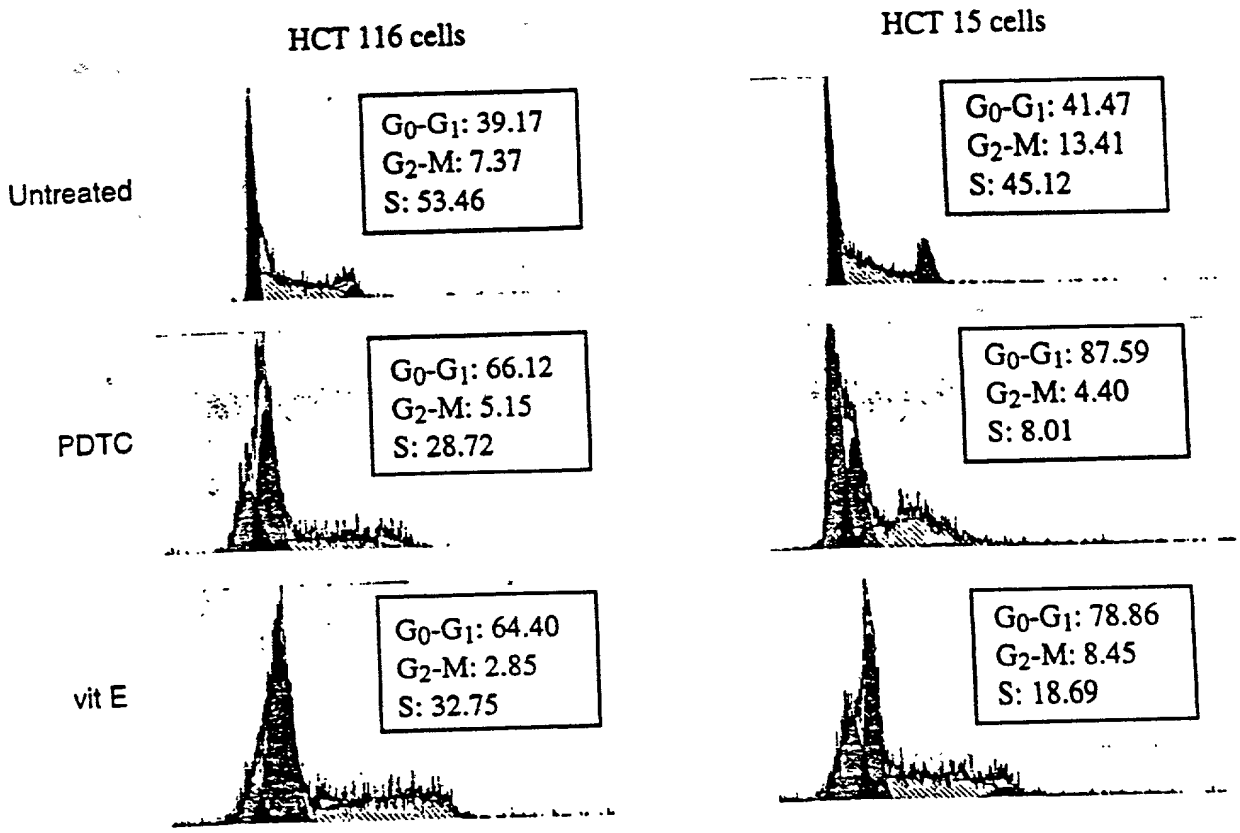

HCT 116 cells
HCT 15 cells
Untreated
PDTC
vit E
G0-G1: 39.17
G2-M: 7.37
S: 53.46
G0-G1: 41.47
G2-M: 13.41
S: 45.12
G0-G1: 66.12
G2-M: 5.15
S: 28.72
G0-G1: 87.59
G2-M: 4.40
S: 8.01
G0-G1: 64.40
G2-M: 2.85
S: 32.75
G0-G1: 78.86
G2-M: 8.45
S: 18.69

FIGURE 1B

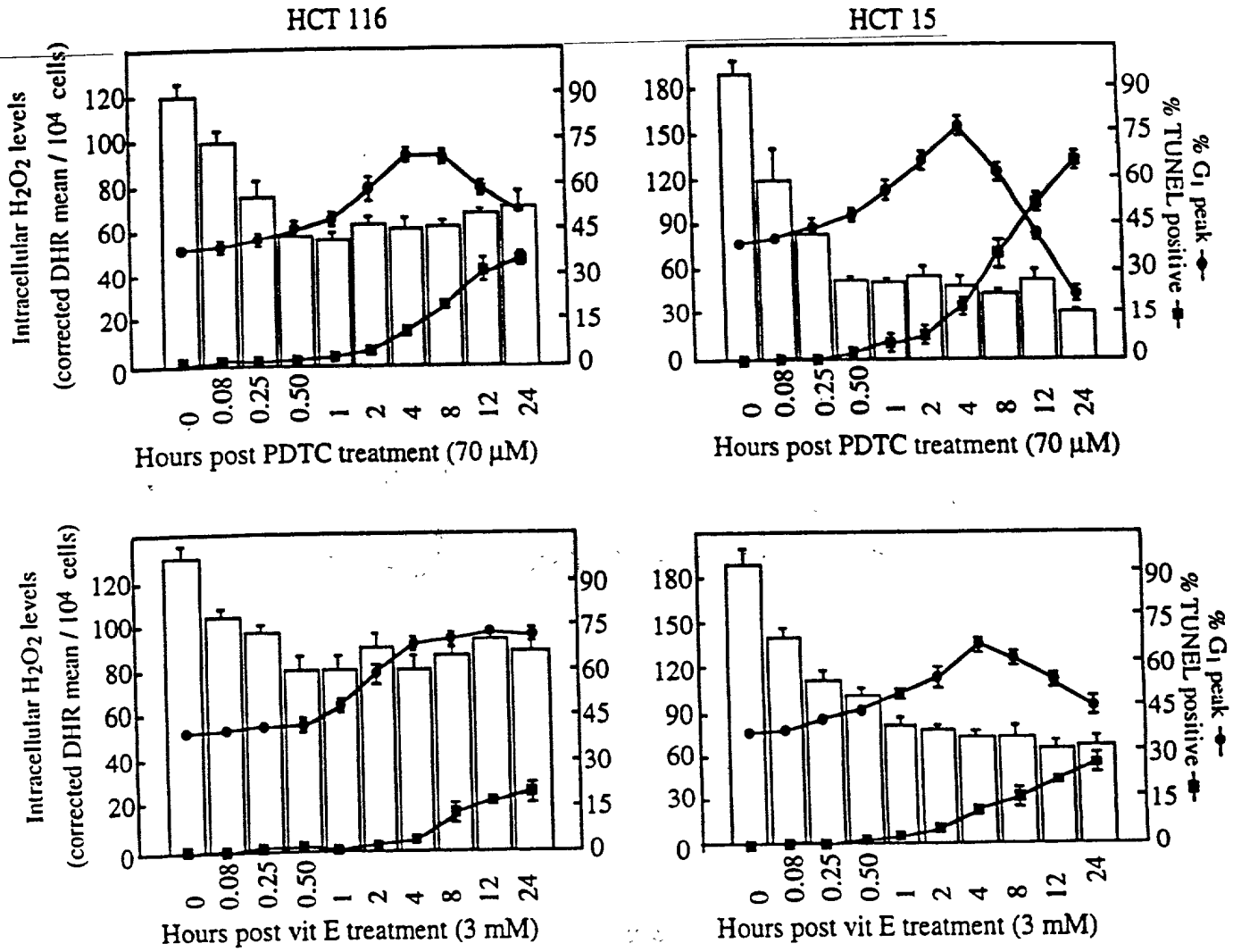

HCT 116
HCT 15
Intracellular H_2O_2 levels
(corrected DHR mean / 10^4 cells)
% G_1 peak
% TUNEL positive
Hours post PDTC treatment (70 μM)
Hours post vit E treatment (3 mM)
0
0.08
0.25
0.50
1
2
4
8
12
24

FIGURE 1C

Figure 1D

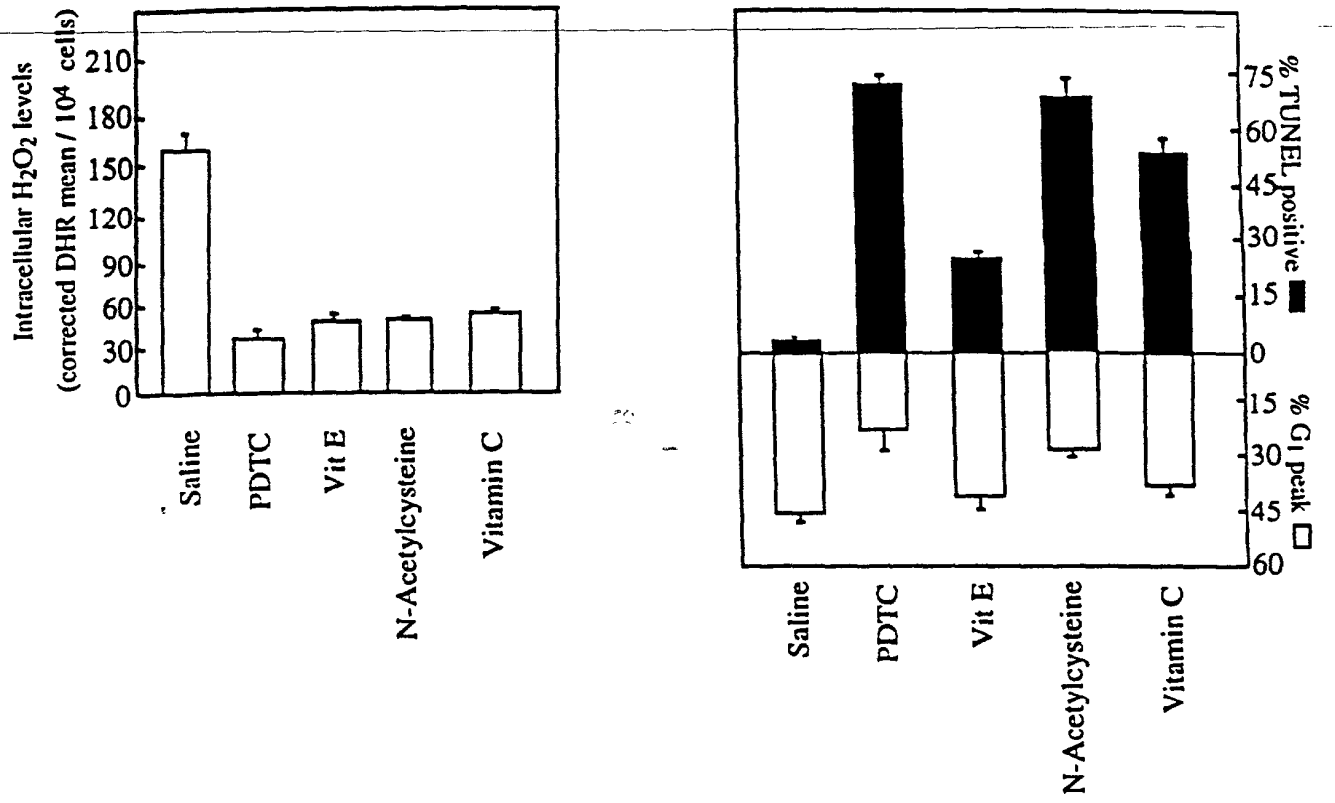


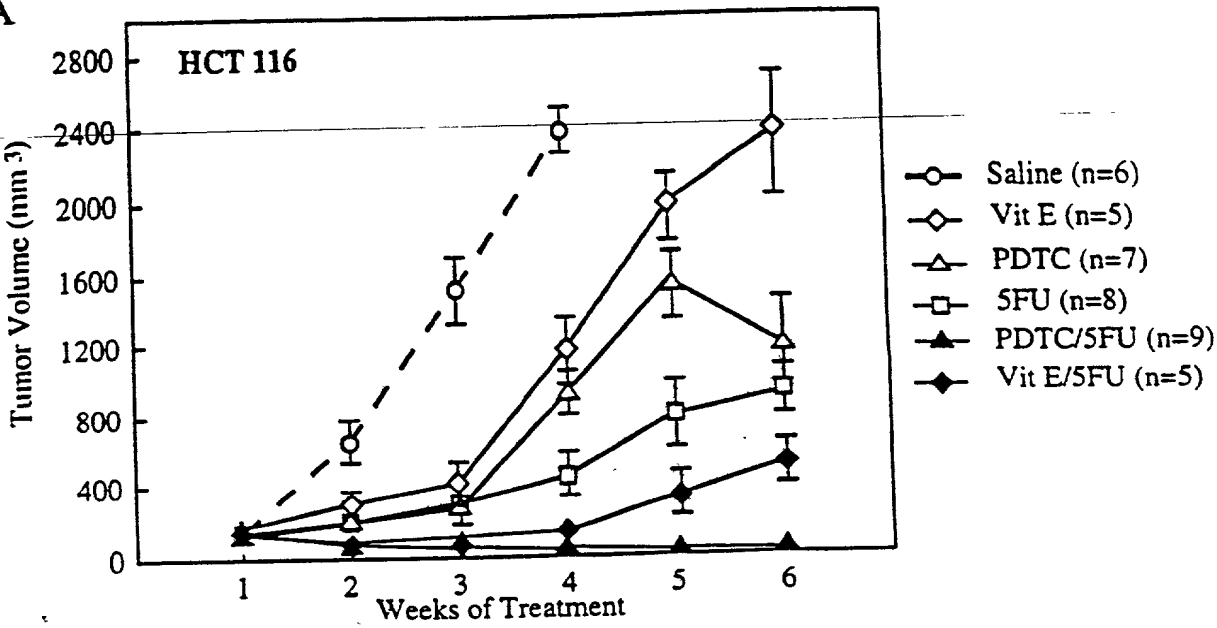
Figure 1E

Sensitization of HCT 116 and HCT 15 colon cancer cells to chemotherapeutic agents by PDTC (70 μM) or vitamin E (3 mM)

Cell line	Drug	IC_{50} (μM)[a]		
		- Antioxidant	+PDTC	+vitamin E
HCT 116	5FU	3.8 (±0.21)	1.5 (±0.29)	1.7 (±0.20)
	Doxorubicin	0.32 (±0.07)	0.09 (±0.08)	0.13 (±0.05)
HCT 15	5FU	11.4 (±0.11)	1.01 (±0.09)	1.4 (±0.10)
	Doxorubicin	1.51 (±0.07)	0.11 (±0.05)	0.17 (±0.04)

[a]The concentration of 5-FU or doxorubicin required to reduce soft agar colony formation by 50% (±s.e.m.). Underscored: signficantly different from -antioxidant group ($P<0.01$), as determined by analysis of variance with multiple comparison adjustment.

Figure 2A



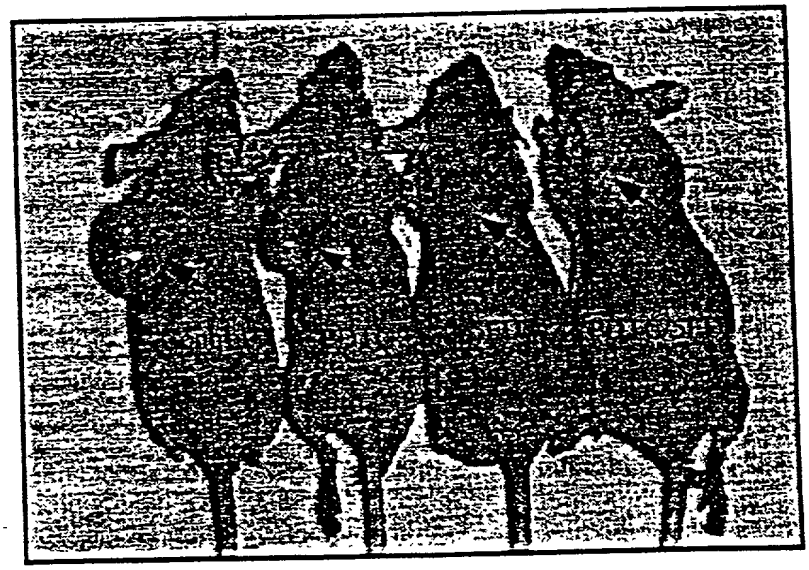


Figure 2B

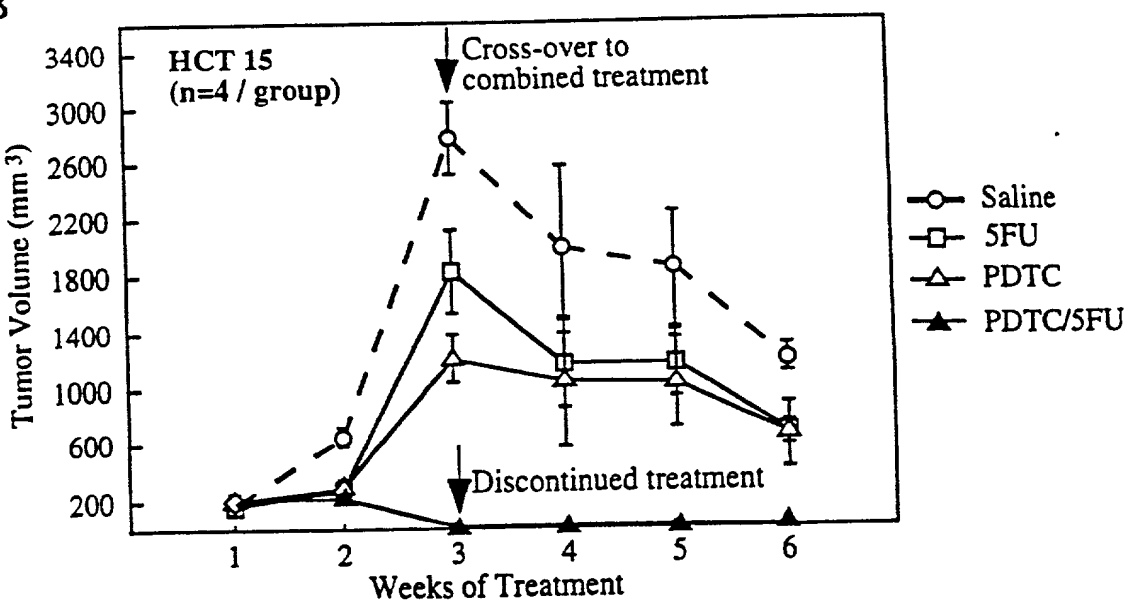


Figure 3A Western blot

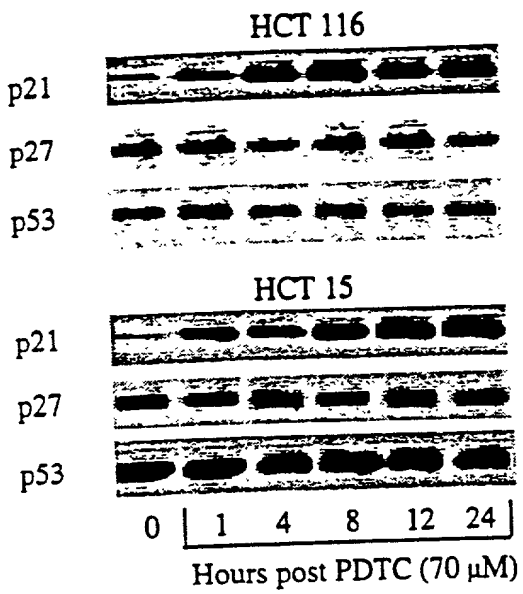


Figure 3B Northern blot

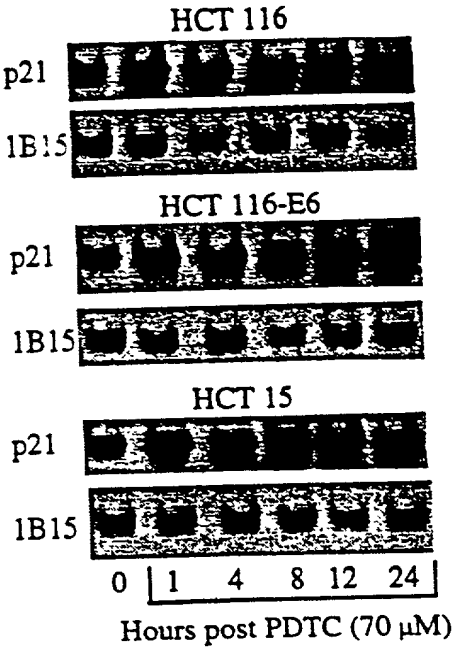
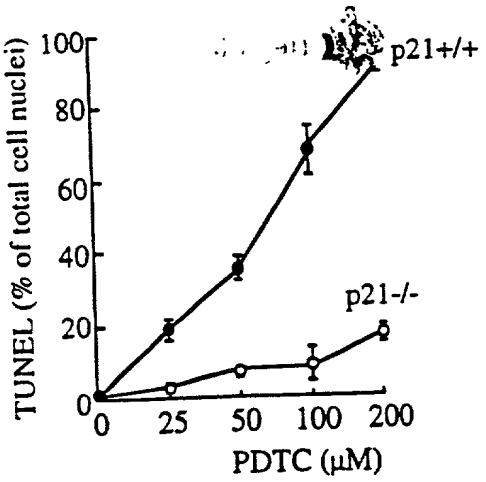


Figure 3C



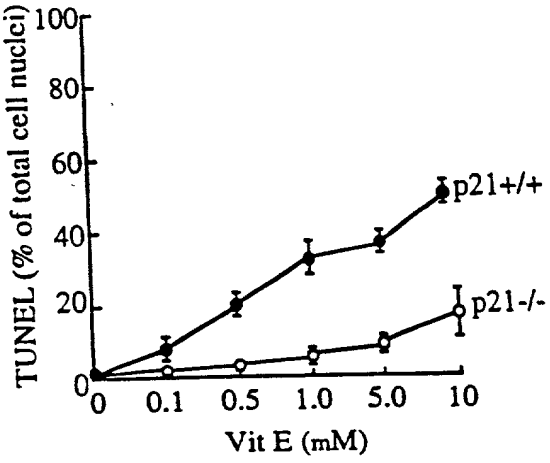


Figure 4A

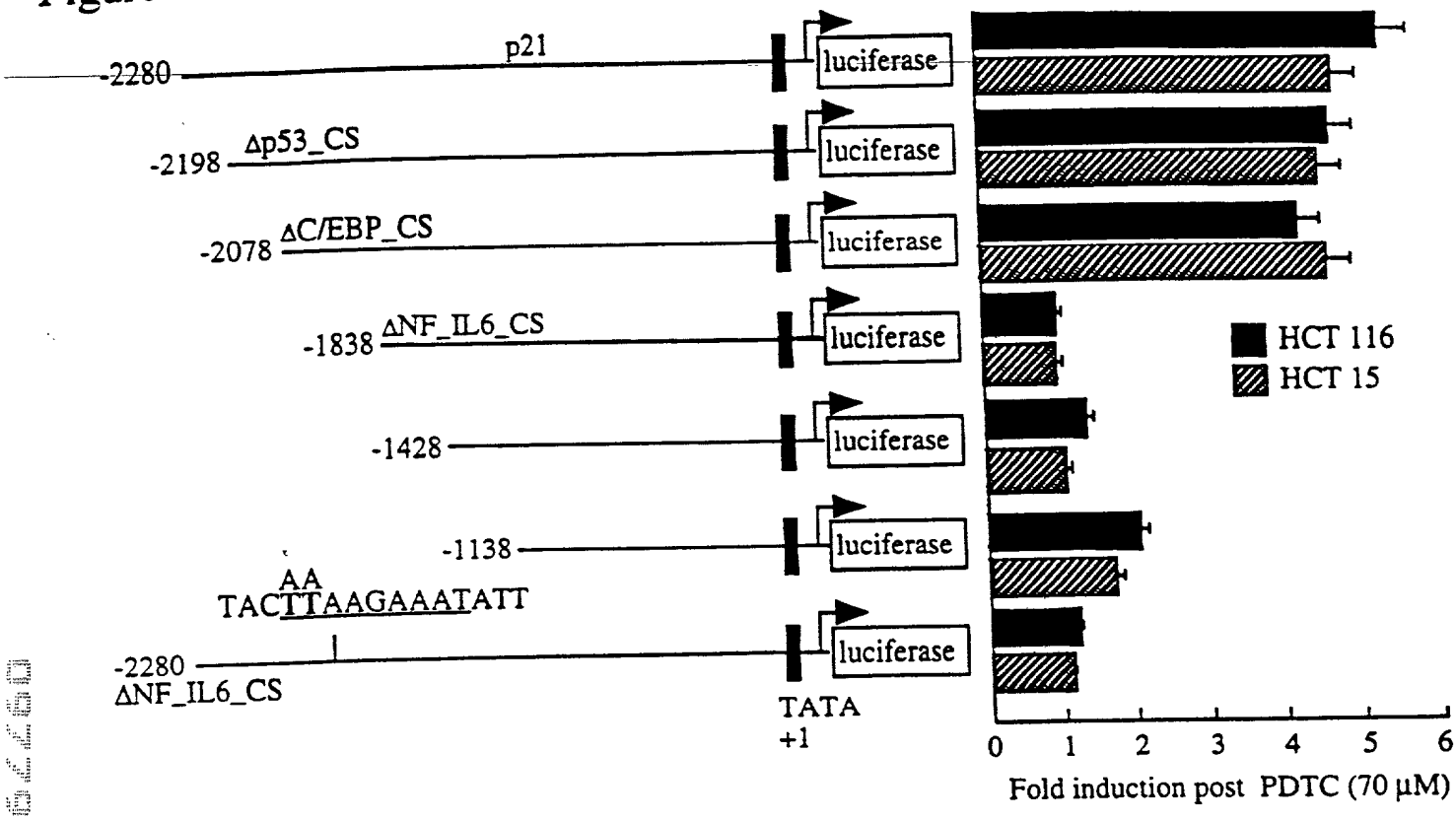

p21
-2280
luciferase
Δp53_CS
-2198
luciferase
ΔC/EBP_CS
-2078
luciferase
ΔNF_IL6_CS
-1838
luciferase
-1428
luciferase
-1138
luciferase
AA
TACTTAAGAAATATT
-2280
ΔNF_IL6_CS
luciferase
TATA
+1
HCT 116
HCT 15
0
1
2
3
4
5
6
Fold induction post PDTC (70 μM)

Figure 4B

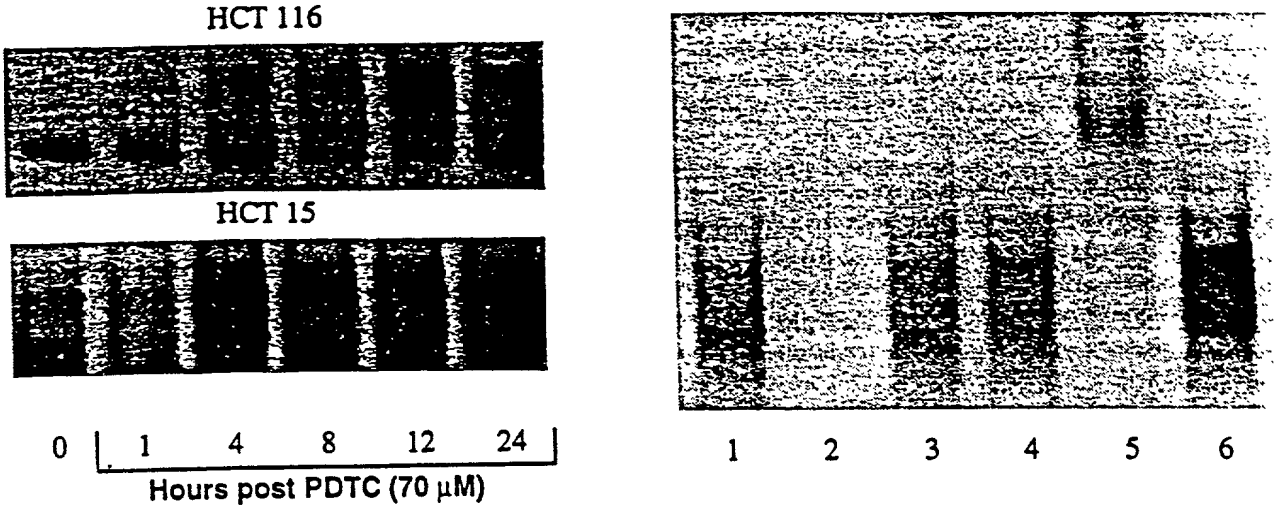


Figure 4C

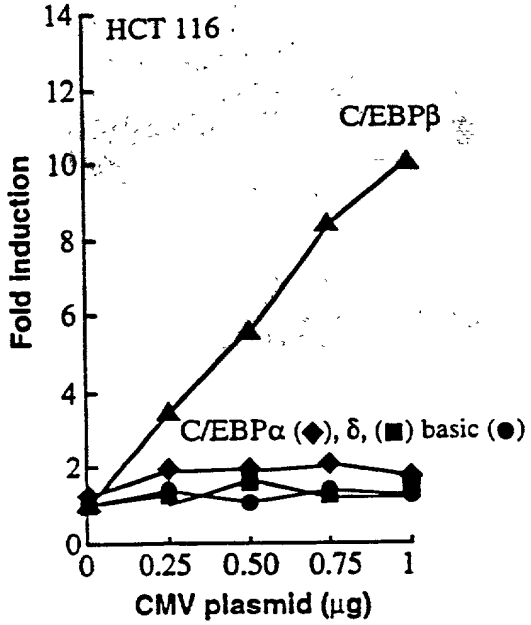


Figure 4D

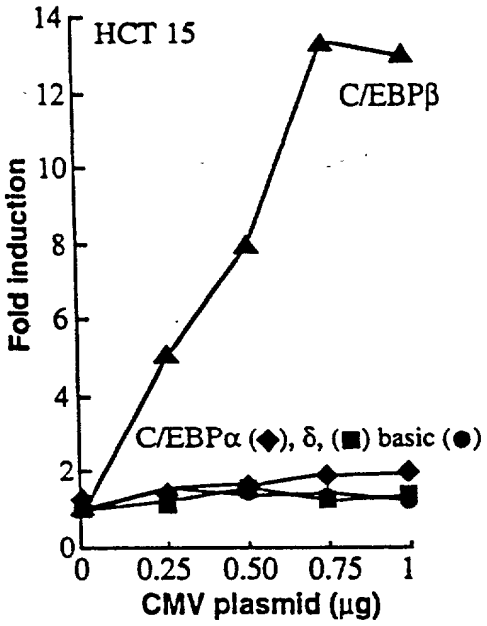


Figure 4E

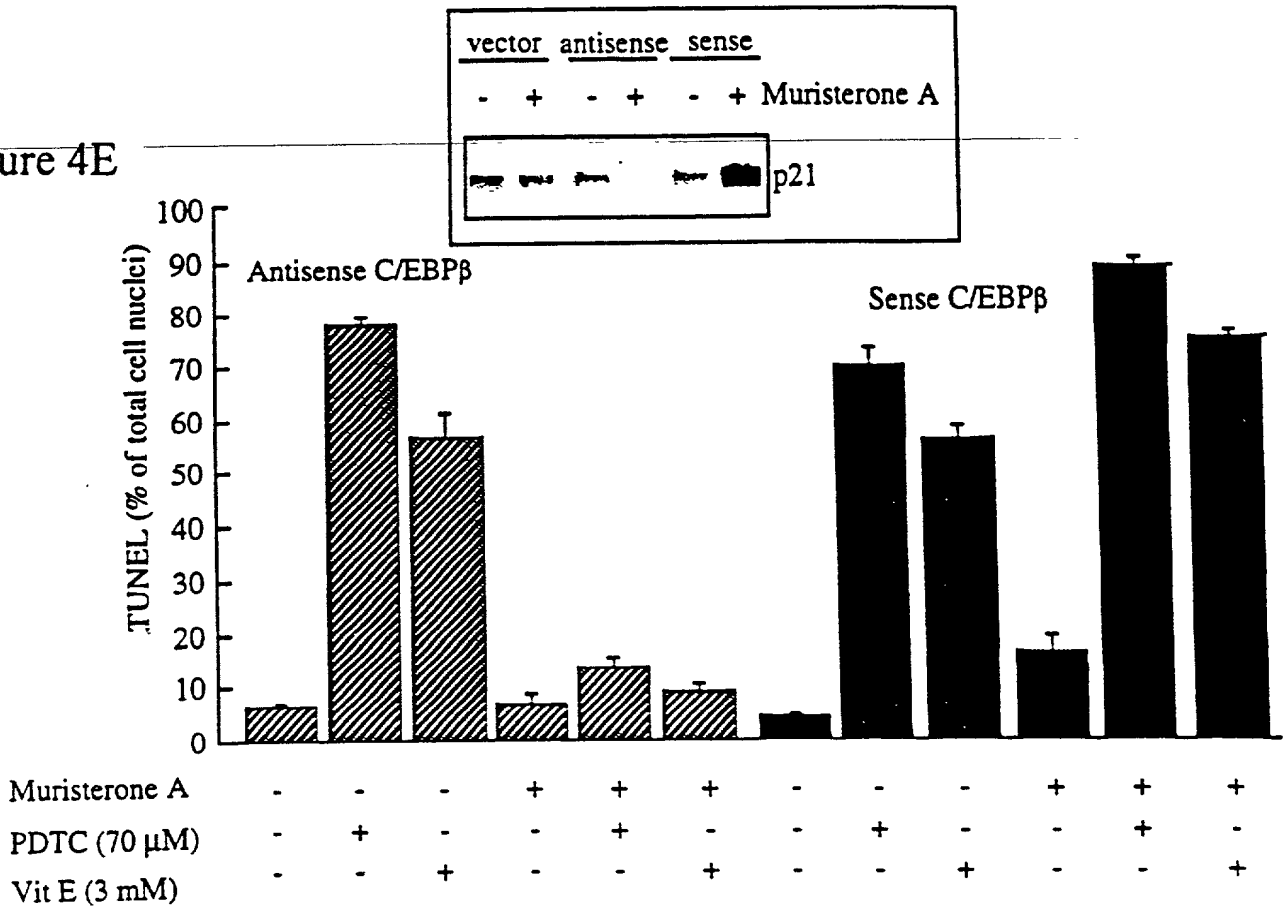
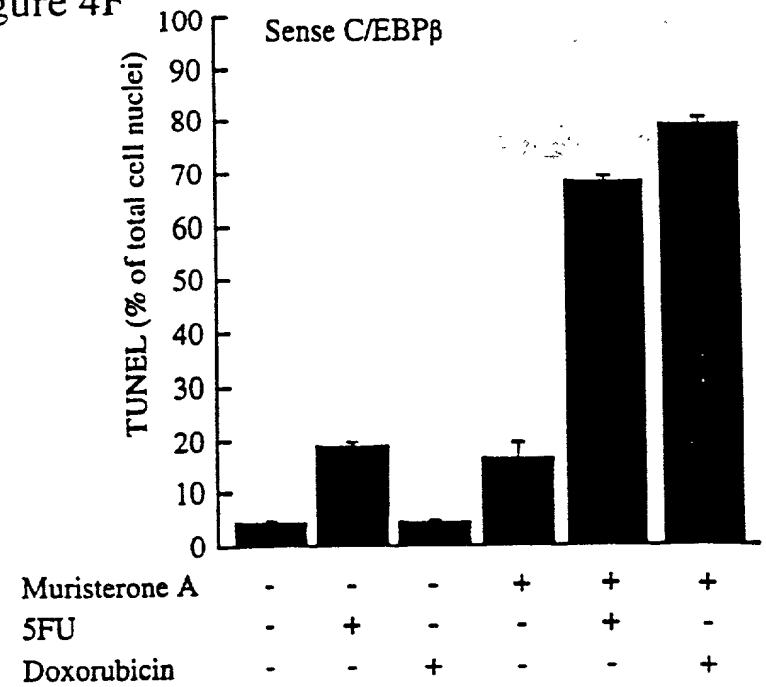


Figure 4F



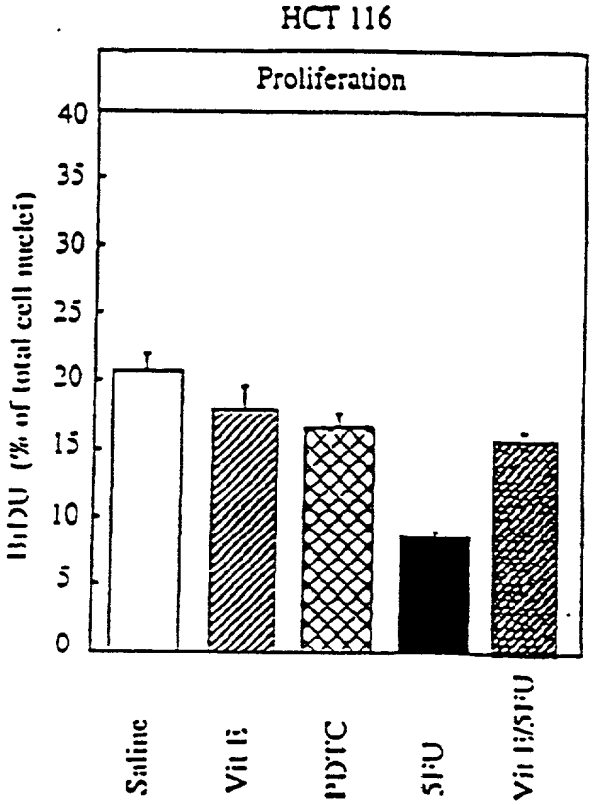

HCT 116
Proliferation
BrDU (% of total cell nuclei)
40
35
30
25
20
15
10
5
0
Saline
PDTC
5FU

Figure 5A

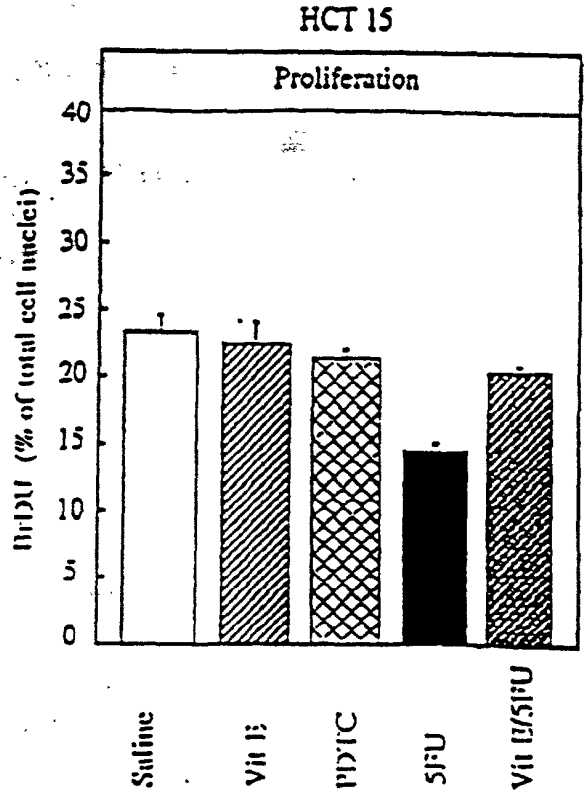

HCT 15
Proliferation
BrDU (% of total cell nuclei)
40
35
30
25
20
15
10
5
0
Saline
Vit E
PDTC
5FU
Vit E/5FU

FIGURE 5B

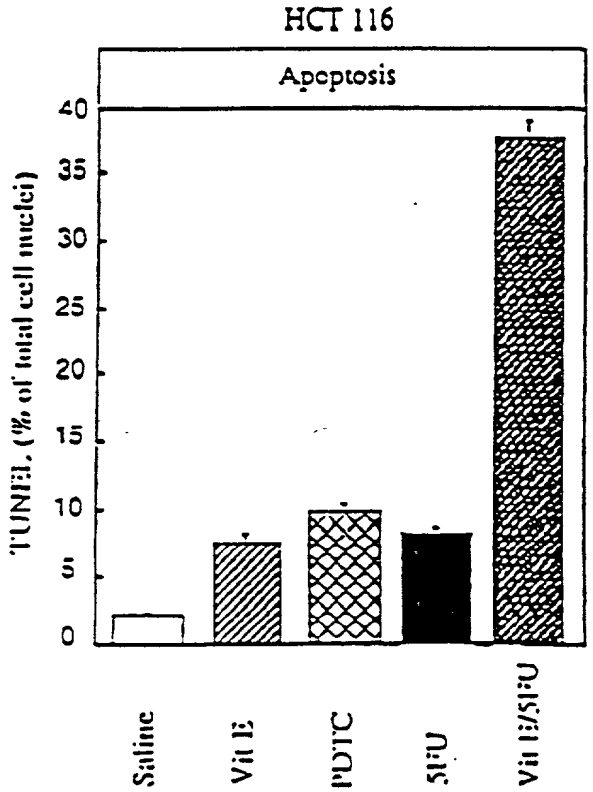

HCT 116
Apoptosis
TUNEL (% of total cell nuclei)
40
35
30
25
20
15
10
5
0
Saline
Vit E
PDTC
5FU
Vit E/5FU

FIGURE 6A

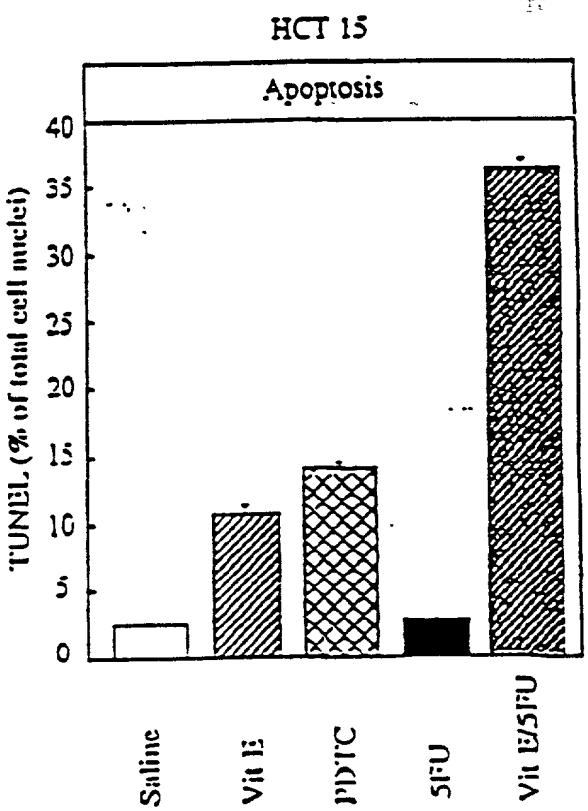

HCT 15
Apoptosis
TUNEL (% of total cell nuclei)
40
35
30
25
20
15
10
5
0
Saline
Vit E
PDTC
5FU
Vit E/5FU

FIGURE 6B

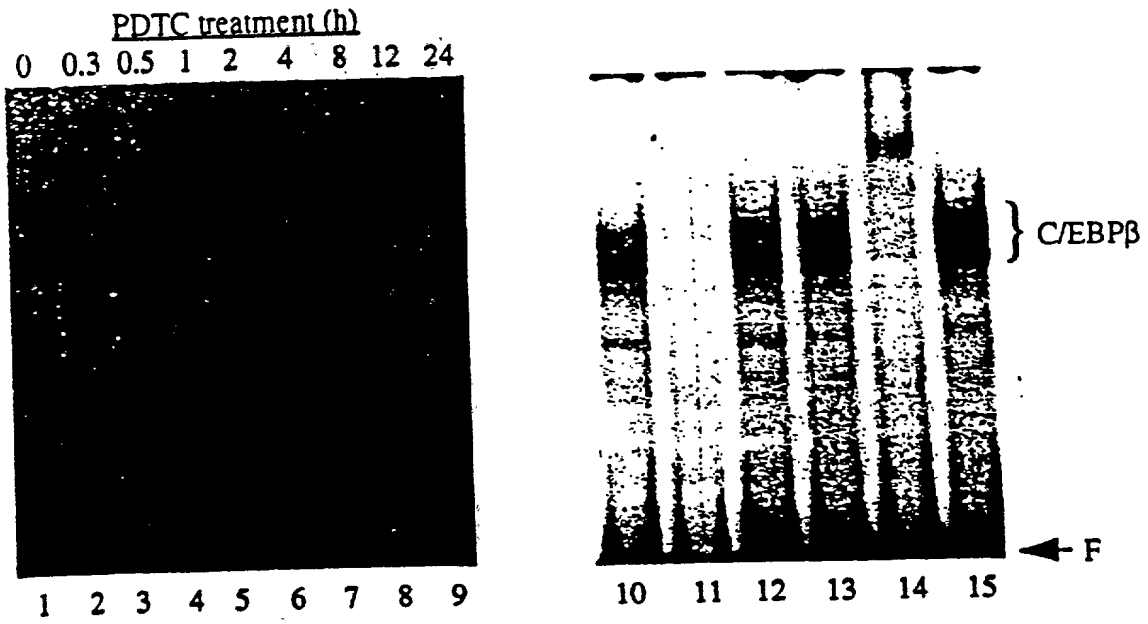

PDTC treatment (h)
0 0.3 0.5 1 2 4 8 12 24
1 2 3 4 5 6 7 8 9
C/EBPβ
F
10 11 12 13 14 15

Figure 7A

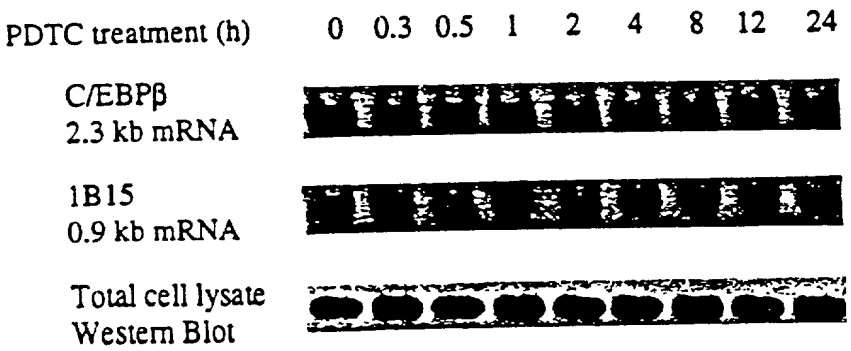

PDTC treatment (h)
0 0.3 0.5 1 2 4 8 12 24
C/EBPβ
2.3 kb mRNA
1B15
0.9 kb mRNA
Total cell lysate
Western Blot

Figure 7B

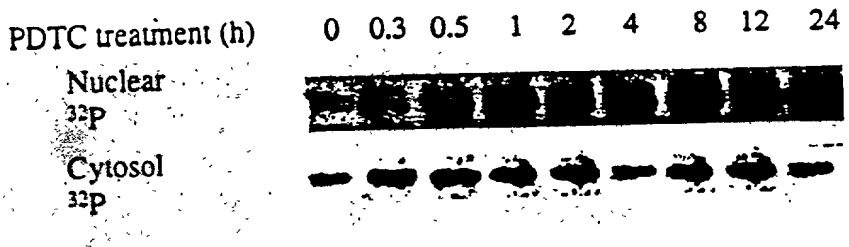
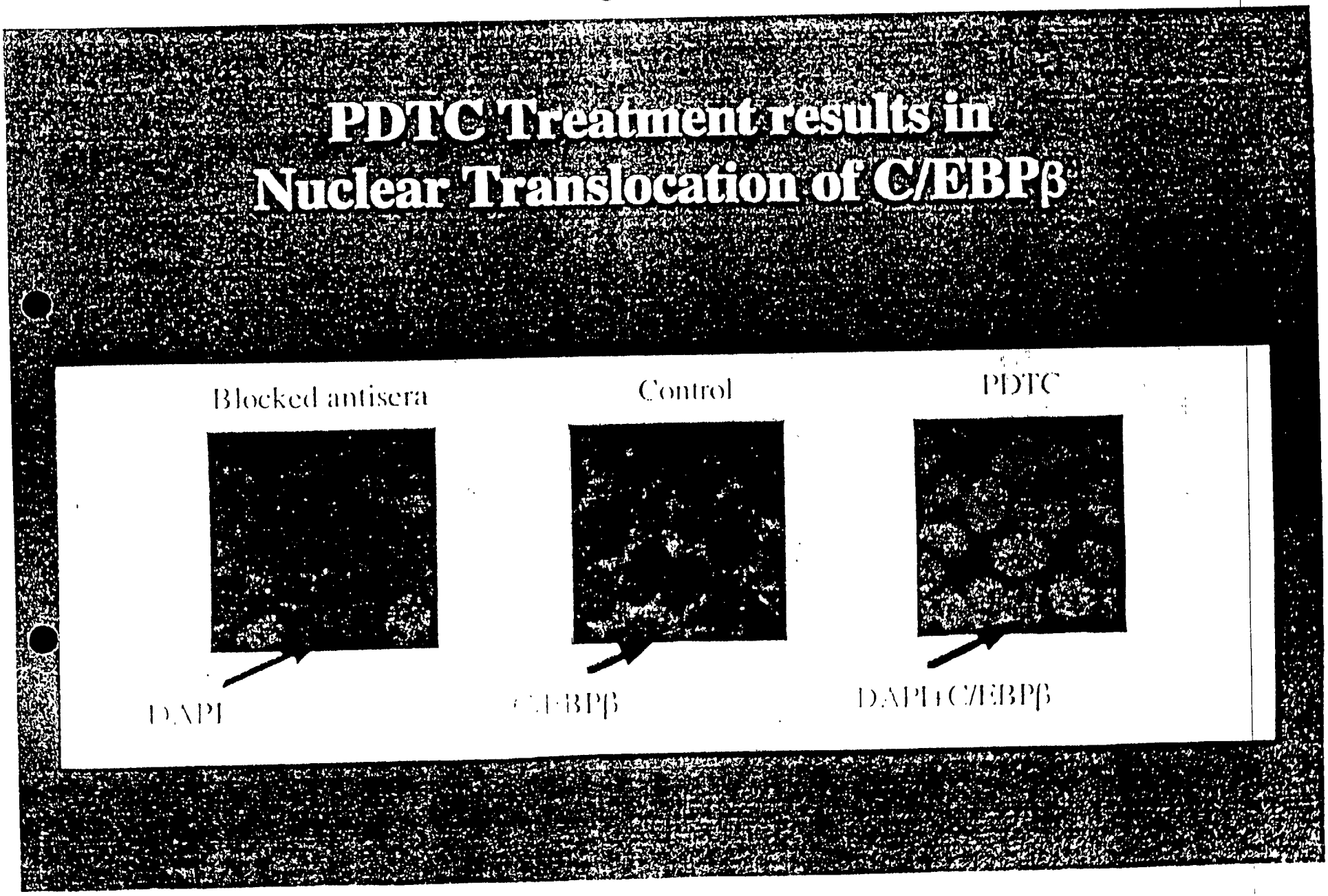


Figure 7C

Figure 7D


PDTC Treatment results in
Nuclear Translocation of C/EBPβ
Blocked antisera
Control
PDTC
DAPI
C/EBPβ
DAPI+C/EBPβ

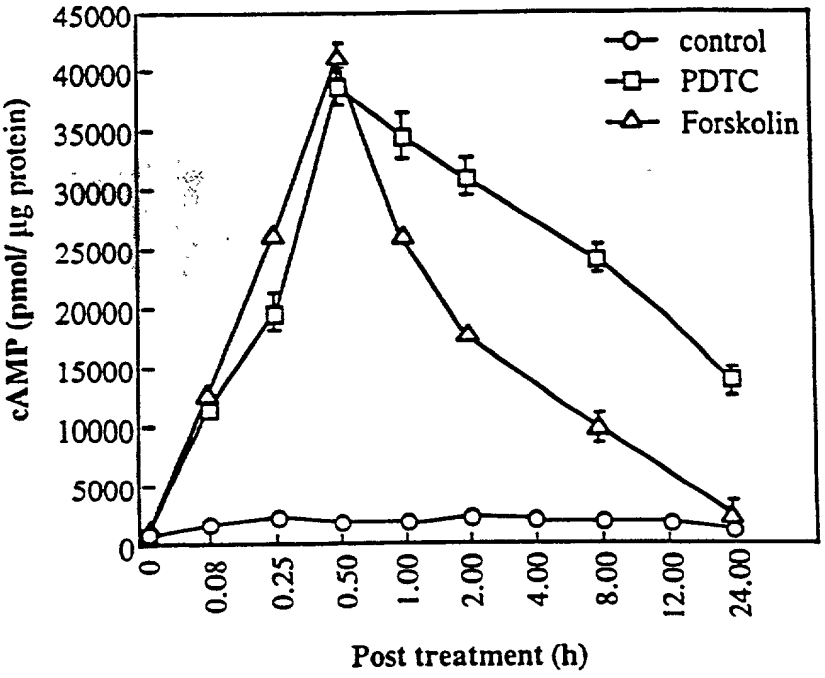

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
cAMP (pmol/ µg protein)
control
PDTC
Forskolin
0
0.08
0.25
0.50
1.00
2.00
4.00
8.00
12.00
24.00
Post treatment (h)

FIGURE 8A

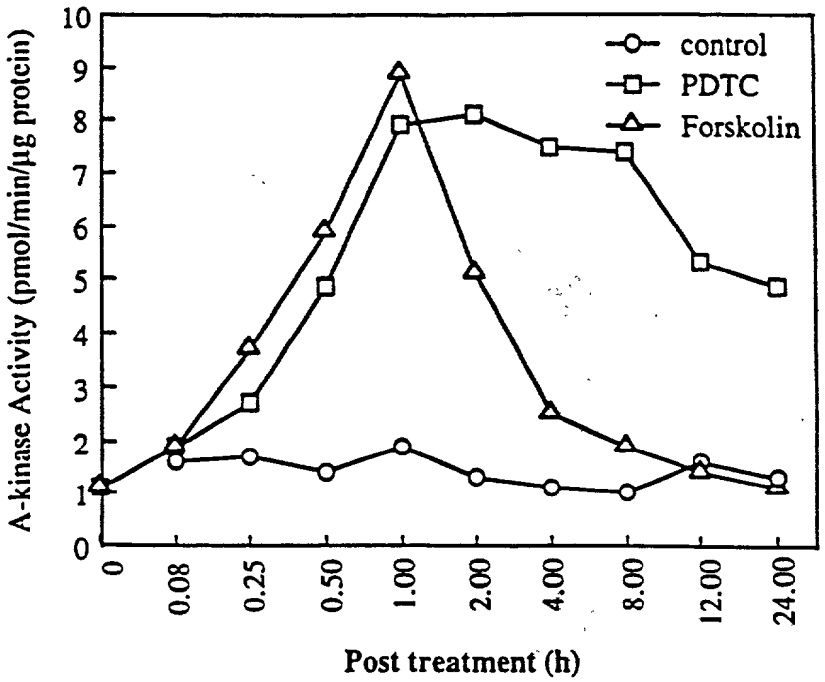

A-kinase Activity (pmol/min/μg protein)
10
9
8
7
6
5
4
3
2
1
0
control
PDTC
Forskolin
0
0.08
0.25
0.50
1.00
2.00
4.00
8.00
12.00
24.00
Post treatment (h)

FIGURE 8B

Figure 9A

Figure 9B Trypsin cleavage

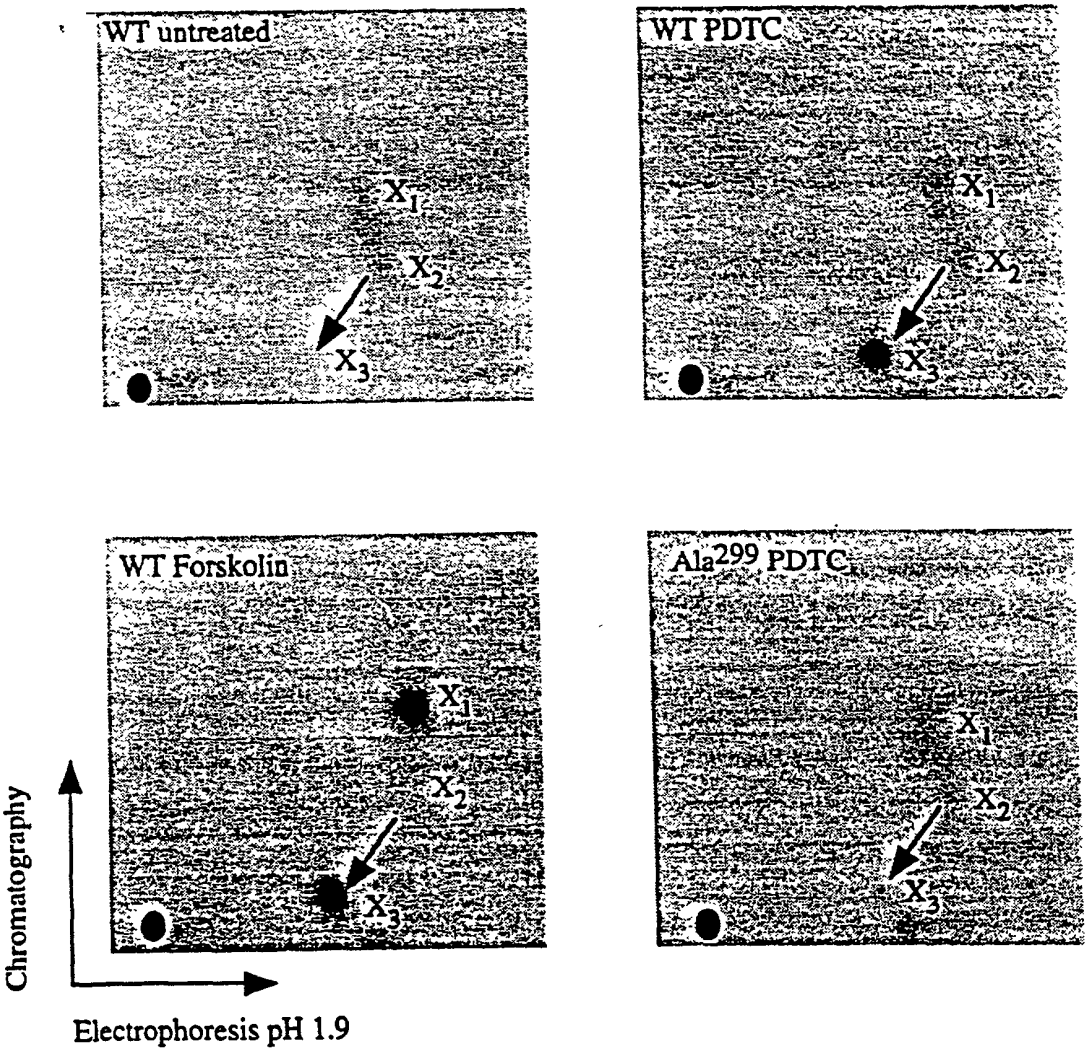


Figure 9C

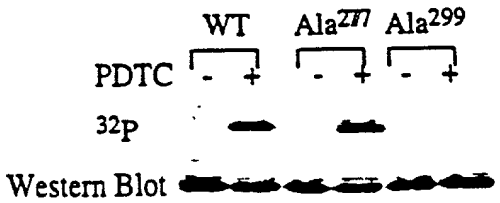


Figure 10A

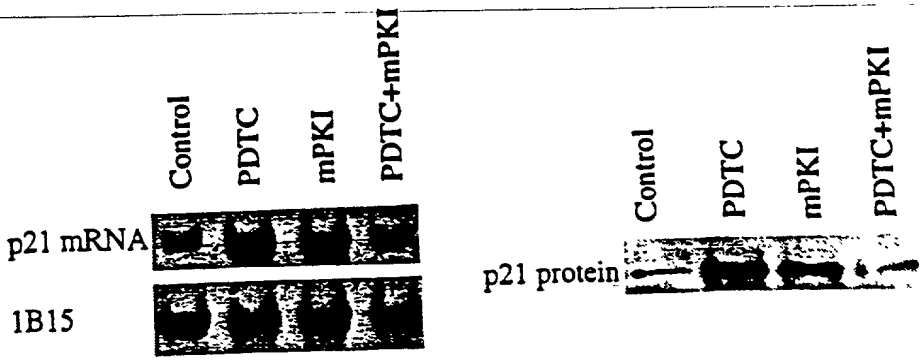


Figure 10B

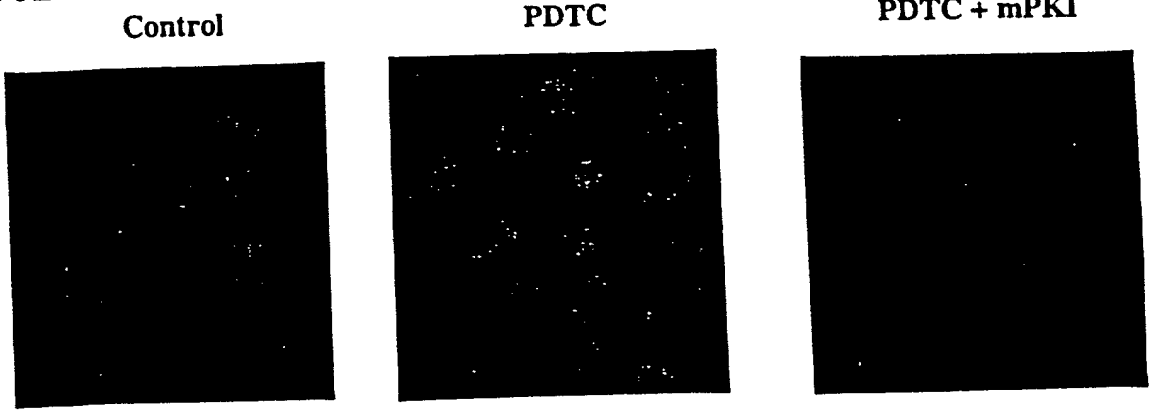
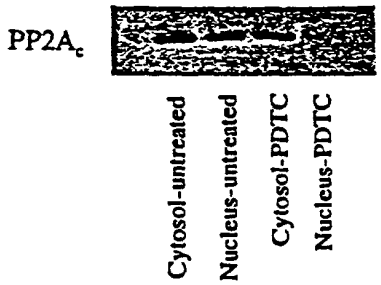


FIGURE 11

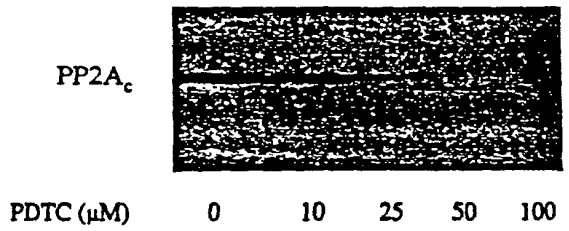
Carboxylmethylation of PP2Ac is Inhibited by Antioxidants



DKO-1 cells were incubated in serum-containing media containing [methyl-^{3}H]S-adenosyl methionine and/or 70μM PDTC for 3 hours. Cytosolic or nuclear fractions were prepared and C/EBPβ immunoprecipitated using standard methods. Antibody/antigen complexes were resolved by SDS-PAGE and the presence of PP2Ac was detected by fluorography (overnight).

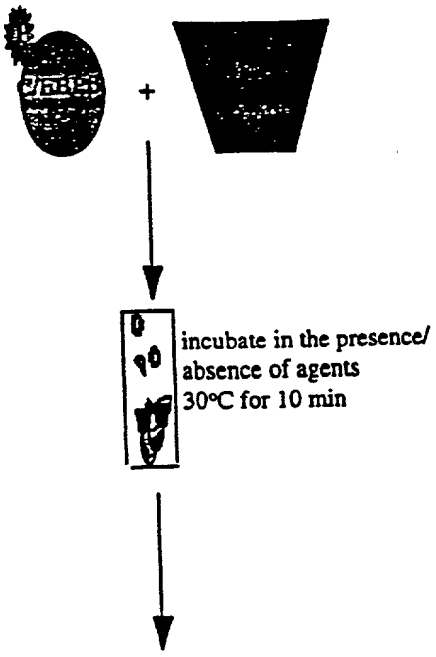
FIGURE 12

Antioxidants Inhibit Methyltransferase Activity Against PP2Ac



PP2A$_{A/C}$ was incubated in the presence of [methyl]^{3}H]S-adenosyl methionine, increasing concentrations of PDTC and partially purified rat methyltransferase for 30 min at 37C. The reaction was terminated by the addition of SDS-sample buffer. Samples were resolved by SDS-PAGE and the presence of methylated PP2A dimers visualized by fluorography.

PDTC Inhibits PP2A, but not PP1, Activity



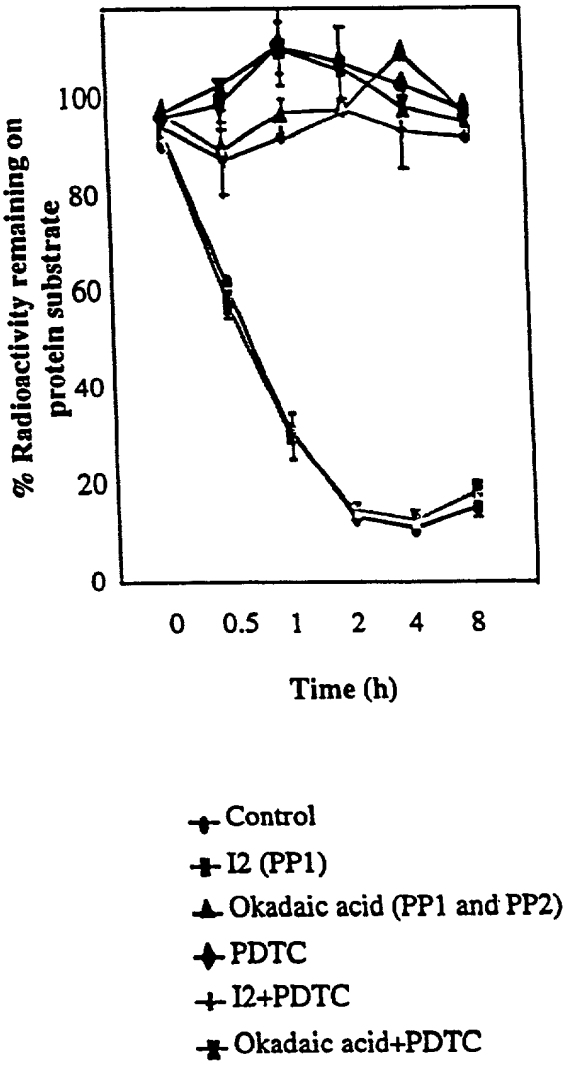


Figure 13

Figure 14 - C/EBPb and PP2ac are components of isolated Methyltransferase activity